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[Continued on next page]

(54) Title: HYBRID AND TANDEM EXPRESSION OF NEISSERIAL PROTEINS

ΔG287-919-His
ΔG287-Orf46.1-His
ΔG287-953-His
ΔG287-961-His
ΔG287-230-His
ΔG287-936-His
ΔG287-287-His
ΔG287-287_{nz}-His
ΔG287-741_{MC58}-His
ΔG287-741_{ET37}-His

961c-741_{MC58}-His
961c-983-His
961c-Orf46.1-His
961cL-741_{MC58}
961cL-287
961c-230-His
961c-936-His

Orf46.1-287-His
Orf46.1-919-His
Orf46.1-741_{MC58}-His
Orf46.1-961-His
Orf46.1-961c-His
Orf46.1-983-His
Orf46.1-936-His
Orf46.1-230-His

ΔG287$_{nz}$-919-His
ΔG287$_{nz}$-953-His
ΔG287$_{nz}$-961-His
ΔG287$_{nz}$-287-His
ΔG287$_{nz}$-287$_{nz}$-His
ΔG287$_{nz}$-741$_{MC58}$-His

ΔG741$_{MC58}$-961c-His
ΔG741$_{MC58}$-961-His
ΔG741$_{MC58}$-983-His
ΔG741$_{MC58}$-Orf46.1-His
ΔG741$_{MC58}$-741$_{MC58}$-His
ΔG741$_{MC58}$-741$_{ET37}$-His

230-741_{MC58}-His
230-Orf46.1-His
230-961-His
230-961c-His
936-741_{MC58}-His
936-Orf46.1-His
936-961-His
936-741_{ET37}-His

ΔG287-919-Orf46.1-His
ΔG287-Orf46.1-919-His
919-287-Orf46-His
Orf46.1-287-919-His

919-287
953-287
919-Orf46.1-His

ΔG983-741_{MC58}-His
ΔG983-961c-His
ΔG983-961-His
ΔG983-Orf46.1-His

(57) Abstract: Two or more Neisserial proteins are joined such that they are translated as a single polypeptide chain. Hybrid proteins are represented by the formula NH_2-A-[-X-L-]$_n$-B-COOH where X is an amino acid sequence, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and *n* is an integer greater than I. Proteins where each of the *n* -X- moieties shares sequence identity to each other -X- moiety, the protein is a 'tandem protein'.

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HYBRID AND TANDEM EXPRESSION OF NEISSERIAL PROTEINS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of protein expression. In particular, it relates to the expression of proteins from *Neisseria* (*e.g. N.gonorrhoeae* or, preferably, *N.meningitidis*).

BACKGROUND ART

References 1 and 2 disclose alternative and improved approaches for the expression of the Neisserial proteins disclosed in references 3 to 6. One such method is to produce 'hybrid' proteins in which two or more Neisserial proteins are expressed as a single polypeptide chain. This approach offers two advantages. First, a protein that may be unstable or poorly expressed on its own can be assisted by adding a suitable hybrid partner that overcomes the problem. Second, commercial manufacture is simplified as only one expression and purification need be employed in order to produce two separately-useful proteins.

It is an object of the present invention to provide further alternative and improved approaches for the expression of Neisserial proteins.

DISCLOSURE OF THE INVENTION

Hybrid proteins

Thus the invention provides a method for the simultaneous expression of two or more (*e.g.* 3, 4, 5, 6 or more) Neisserial proteins, in which said two or more proteins are joined such that they are translated as a single polypeptide chain. In general, the hybrid proteins of the invention can be represented by the formula: NH_2-A-[-X-L-]$_n$-B-COOH

wherein X is an amino acid sequence, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and n is an integer greater than 1.

The value of n is between 2 and x, and the value of x is typically 3, 4, 5, 6, 7, 8, 9 or 10. Preferably n is 2, 3 or 4; it is more preferably 2 or 3; most preferably, $n = 2$.

The -X- moieties

There are two main groups of hybrid proteins according to the invention. These two groups are not mutually exclusive.

In the first group, each -X- moiety is:

(a) an orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, 961 or 983 amino acid sequence;

(b) an amino acid sequence having sequence identity to an amino acid sequence from (a); or

(c) an amino acid sequence comprising a fragment of an amino acid sequence from (a).

A preferred subset of (a) is: orf46.1, 230, 287, 741, 919, 936, 953, 961 and 983. A more preferred subset of (a) is: orf46.1, 287, 741 and 961. Figure 3 shows preferred hybrid proteins.

In the second group, the hybrid protein comprises a first -X- moiety ($-X_a-$) and a second -X- moiety ($-X_b-$). The $-X_a-$ moiety has one of the following amino acid sequences:

(d) the 446 even SEQ IDs (*i.e.* 2, 4, 6, ... , 890, 892) disclosed in reference 3.

(e) the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference 4;

(f) the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241, disclosed in reference 5;

(g) the 2160 amino acid sequences NMB0001 to NMB2160 from reference 7; or

(h) an amino acid sequence disclosed in reference 1 or reference 2.

The $-X_b-$ moiety is related to $-X_a-$ such that: (i) $-X_b-$ has sequence identity to $-X_a-$, and/or (j) $-X_b-$ comprises a fragment of $-X_a-$.

Examples of this second type of hybrid protein include proteins in which two or more -X- moieties are identical, or in which they are variants of the same protein *e.g.* two polymorphic forms of the same protein may be expressed as $-X_a-X_b-$, and three polymorphic forms may be expressed as $-X_a-X_b-X_c-$ *etc.*

The $-X_a-$ and $-X_b-$ moieties may be in either order from N-terminus to C-terminus.

The $-X_a-$ moiety is preferably an orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, 961 or 983 amino acid sequence. The $-X_a-$ moiety is more preferably an orf46.1, 230, 287, 741, 919, 936, 953, 961 or 983 amino acid sequence. The $-X_a-$ moiety is most preferably an orf46.1, 287, 741 or 961 amino acid sequence.

In proteins where each of the n -X- moieties shares sequence identity to each other -X- moiety, the protein is referred to as a 'tandem protein'. Tandem proteins in which n=2 are preferred.

The degree of 'sequence identity' referred to in (b) and (i) is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more, up to 100%). This includes mutants, homologs, orthologs, allelic variants *etc.* [*e.g.* see ref. 8]. Identity is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*. Typically, 50% identity or more between two proteins is considered as an indication of functional equivalence.

The 'fragment' referred to in (c) and (j) should consist of least m consecutive amino acids from an amino acid sequence from (a), (d), (e), (f), (g) or (h) and, depending on the particular sequence, m is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 150, 200 or more).

Preferably the fragment comprises an epitope from an amino acid sequence from (a), (d), (e), (f), (g) or (h). Preferred fragments are those disclosed in references 9 and 10.

Preferred (c) and (j) fragments are C- and/or N-terminal truncations (*e.g.* Δ1-287, Δ2-287 *etc.*).

Preferred (b), (c), (i) and (j) sequences omit poly-glycine sequences. This has been found to aid expression [ref. 2]. Poly-glycine sequences can be represented as $(Gly)_g$, where $g \geq 3$ (*e.g.* 4, 5, 6, 7, 8, 9 or more). If a -X- moiety includes a poly-glycine sequence in its wild-type form, it is preferred to omit this sequence in the hybrid proteins of the invention. This may be by disrupting or removing the $(Gly)_g$ – by deletion (*e.g.* CGGGGS→ CGGGS, CGGS, CGS or CS), by substitution (*e.g.* CGGGGS→ CGXGGS, CGXXGS, CGXGXS *etc.*), and/or by insertion (*e.g.* CGGGGS→ CGGXGGS, CGXGGGS, *etc.*). Deletion of $(Gly)_g$ is preferred, and deletion of the N-terminus portion of a protein up to and including the poly-glycine sequence (*e.g.* deletion of residues 1-32 in SEQ ID 1) is referred to herein as 'ΔG'. Poly-glycine omission is particularly useful for proteins 287, 741, 983 and Tbp2 (ΔG287, ΔG741, ΔG983 and ΔGTbp2 – references 1 & 2).

Preferred (c) and (j) fragments omit complete protein domains. This is particularly useful for protein 961, 287, and ORF46. Once a protein has been notional divided into domains, (c) and (j) fragments can omit one or more of these domains (*e.g.* 287B, 287C, 287BC, $ORF46_{1-433}$, $ORF46_{434-608}$, 961c – reference 2; Figures 4 and 5 herein).

287 protein has been notionally split into three domains, referred to as A, B & C (see Figure 5 of reference 2). Domain B aligns with IgA proteases, domain C aligns with transferrin-binding proteins, and domain A shows no strong alignment with database sequences. An alignment of polymorphic forms of 287 is disclosed in reference 8.

ORF46 has been notionally split into two domains – a first domain (amino acids 1-433; ORF46.1) which is well-conserved between species and serogroups, and a second domain (amino acids 434-608) which is not well-conserved. The second domain is preferably deleted, leaving ORF46.1. An alignment of polymorphic forms of ORF46 is disclosed in reference 8.

961 protein has been notionally split into several domains (Figure 4).

If a -X- moiety has a leader peptide sequence in its wild-type form, this may be included or omitted in the hybrid proteins of the invention. Where the leader peptide is omitted, this is a preferred example of an amino acid sequence within (c) and (j). In one embodiment, the leader peptides will be deleted except for that of the -X- moiety located at the N-terminus of the hybrid protein *i.e.* the leader peptide of X_1 will be retained, but the leader peptides of X_2 ... X_n will be omitted. This is equivalent to deleting all leader peptides and using the leader peptide of X_1 as moiety -A-.

When n=2, preferred pairs of -X- moieties are: ΔG287 and 230; ΔG287 and 936; ΔG287 and 741; 961c and 287; 961c and 230; 961c and 936; 961cL and 287; 961cL and 230; 961cL and 936;

ORF46.1 and 936; ORF46.1 and 230; 230 and 961; 230 and 741; 936 and 961; 936 and 741. When *n*=2, preferred pairs of -X- moieties for tandem proteins are: ΔG741 and 741; ΔG287 and 287. More specifically, the following combinations of X_1 and X_2 are preferred when *n*=2:

X_1	X_2
ΔG287	230
ΔG287	936
ΔG287	741
ΔG287	961
ΔG287	ORF46.1
ΔG287	919
ΔG287	953
961c	287
961c	230
961c	936
961c	741
961c	983
961c	ΔG983
961c	ORF46.1
961	ORF46.1
961cL	287
961cL	230
961cL	936
ORF46.1	936
ORF46.1	230
ORF46.1	741
ORF46.1	ΔG741
ORF46.1	983
ORF46.1	ΔG983
230	961
230	741
230	ΔG741
936	961
936	741
936	ΔG741
ΔG741	741
ORF46.1	983
ΔG741	ORF46.1
ΔG741	983
ΔG741	961
ΔG741	961c
ΔG983	ORF46.1
ΔG983	961
ΔG983	961c

X_1	X_2
230	ΔG287
936	ΔG287
741	ΔG287
961	ΔG287
ORF46.1	ΔG287
919	ΔG287
953	ΔG287
287	961c
230	961c
936	961c
741	961c
983	961c
ΔG983	961c
ORF46.1	961c
ORF46.1	961
287	961cL
230	961cL
936	961cL
936	ORF46.1
230	ORF46.1
741	ORF46.1
ΔG741	ORF46.1
983	ORF46.1
ΔG983	ORF46.1
961	230
741	230
ΔG741	230
961	936
741	936
ΔG741	936
ΔG287	287
983	ORF46.1
ORF46.1	ΔG741
983	ΔG741
961	ΔG741
961c	ΔG741
ORF46.1	ΔG983
961	ΔG983
961c	ΔG983

Where 287 is used in full-length form, it is preferably at the C-terminal end of a hybrid protein; if it is to be used at the N-terminus, if is preferred to use a ΔG form of 287. Similarly, Where 741 is used

in full-length form, it is preferably at the C-terminal end of a hybrid protein; if it is to be used at the N-terminus, if is preferred to use a ΔG form of 741.

The -L- moieties

For each *n* instances of [-X-L-], linker amino acid sequence -L- may be present or absent. For instance, when *n*=2 the hybrid may be NH_2-X_1-L_1-X_2-L_2-COOH, NH_2-X_1-X_2-COOH, NH_2-X_1-L_1-X_2-COOH, NH_2-X_1-X_2-L_2-COOH, *etc.*

Linker amino acid sequence(s) -L- will typically be short (*e.g.* 20 or fewer amino acids *i.e.* 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include short peptide sequences which facilitate cloning, poly-glycine linkers (*i.e.* Gly_n where n = 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), and histidine tags (*i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable linker amino acid sequences will be apparent to those skilled in the art. A useful linker is GSGGGG (SEQ ID 27), with the Gly-Ser dipeptide being formed from a *Bam*HI restriction site, thus aiding cloning and manipulation, and the Gly_4 tetrapeptide being a typical poly-glycine linker.

If X_{n+1} is a ΔG protein and L_n is a glycine linker, this may be equivalent to X_{n+1} not being a ΔG protein and L_n being absent.

The -A- moiety

-A- is an optional N-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include leader sequences to direct protein trafficking, or short peptide sequences which facilitate cloning or purification (*e.g.* histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more). Other suitable N-terminal amino acid sequences will be apparent to those skilled in the art. If X_1 lacks its own N-terminus methionine, -A- may be a methionine residue.

The -B- moiety

-B- is an optional C-terminal amino acid sequence. This will typically be short (*e.g.* 40 or fewer amino acids *i.e.* 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1). Examples include sequences to direct protein trafficking, short peptide sequences which facilitate cloning or purification (*e.g.* comprising histidine tags *i.e.* His_n where n = 3, 4, 5, 6, 7, 8, 9, 10 or more), or sequences which enhance protein stability. Other suitable C-terminal amino acid sequences will be apparent to those skilled in the art.

Polymorphic forms of proteins

The invention can use amino acid sequences from any strains of *N.meningitidis*. References to a particular protein (*e.g.* '287', or 'ORF46.1') therefore include that protein from any strain. Sequence variations between strains are included within (b), (c), (i) and (j).

Reference sequences from *N.meningitidis* serogroup B include:

Protein	Reference		Protein	Reference
orf1	Ref. 3, SEQ ID 650		orf4	Ref. 3, SEQ ID 218
orf25	Ref. 3, SEQ ID 684		orf40	Ref. 4, SEQ ID 4
orf46	Ref. 6, SEQ ID 1049		orf83	Ref. 3, SEQ ID 314
NMB1343	Ref. 7, NMB1343		230	Ref. 5, SEQ ID 830
233	Ref. 5, SEQ ID 860		287	Ref. 5, SEQ ID 3104
292	Ref. 5, SEQ ID 1220		594	Ref. 5, SEQ ID 1862
687	Ref. 5, SEQ ID 2282		736	Ref. 5, SEQ ID 2506
741	Ref. 5, SEQ ID 2536		907	Ref. 5, SEQ ID 2732
919	Ref. 5, SEQ ID 3070		936	Ref. 5, SEQ ID 2884
953	Ref. 5, SEQ ID 2918		961	Ref. 5, SEQ ID 940
983	Ref. 7, NMB1969			

Reference 8 discloses polymorphic forms of proteins ORF4, ORF40, ORF46, 225, 235, 287, 519, 726, 919 and 953. Polymorphic forms of 961 are disclosed in references 11 & 12. Any of these polymorphic forms may be used in accordance with the present invention.

The sequence listing herein includes polymorphic forms of proteins 741 (SEQ IDs 1-22) and NMB1343 (SEQ IDs 23-24) which have been identified.

Serogroups and strains

Preferred proteins of the invention comprise -X- moieties having an amino acid sequence found in *N.meningitidis* serogroup B. Within a single protein of the invention, individual -X- moieties may be from one or more strains. Where n=2, for instance, X_2 may be from the same strain as X_1 or from a different strain. Where n=3, the strains might be (i) $X_1=X_2=X_3$ (ii) $X_1=X_2\neq X_3$ (iii) $X_1\neq X_2=X_3$ (iv) $X_1\neq X_2\neq X_3$ or (v) $X_1=X_3\neq X_2$, *etc.*

Within serogroup B, preferred -X- moieties are from strains 2996, MC58, 95N477, or 394/98. Strain 95N477 is sometimes referred to herein as 'ET37', this being its electrophoretic type. Strain 394/98 is sometimes referred to herein as 'nz', as it is a New Zealand strain.

Where a form of 287 is used, this is preferably from strain 2996 or from strain 394/98.

Where a form of 741 is used, this is preferably from serogroup B strains MC58, 2996, 394/98, or 95N477, or from serogroup C strain 90/18311.

Where a form of 961 is used, this is preferably from strain 2996.

Strains are indicated as a subscript *e.g.* 741_{MC58} is protein 741 from strain MC58. Unless otherwise stated, proteins mentioned herein (*e.g.* with no subscript) are from *N.meningitidis* strain 2996, which

can be taken as a 'reference' strain. It will be appreciated, however, that the invention is not in general limited by strain. As mentioned above, general references to a protein (*e.g.* '287', '919' *etc.*) may be taken to include that protein from any strain. This will typically have sequence identity to 2996 of 90% or more (*eg.* 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more).

Domain-based expression of protein 961

References 1 and 2 disclose how a protein can be notionally divided into domains and how the protein can be manipulated based on these domains. The present invention extends the application of this approach to protein 961 (also known as 'NadA' [11,12]).

In *N.meningitidis* serogroup B strain 2996, NadA has 405 amino acids. This protein has notionally been divided into the following nine domains (Figure 4):

Domain name	Amino acids	Domain name	Amino acids
961-1 'L'	1-23	961-6	269-286
961-2	24-87	961-7	287-330
961-3	88-143	961-8	331-350
961-4	144-180	961-9	351-405
961-5	181-268		

This information can be used to locate the same domains in other forms of 961.

These domains have been deleted from 961 in strain 2996 in various ways (Figure 5). Preferred fragments of 961 omit one or more of these nine domains *e.g.* the following:

- 961-2 to 961-5 ('961a')
- 961-6 to 961-9 ('961b')
- 961-1 to 961-8 ('961cL')
- 961-2 to 961-8 ('961c')
- 961-2 to 961-6 and amino acids 287-325 from domain 961-7 ('961d')
- 961-2 to 961-8 and amino acids 351-383 from domain 961-9 ('961Δ1')
- 961-1 to 961-8 and amino acids 351-383 from domain 961-9 ('961Δ1L')
- 961-1 to 961-7 and amino acids 331-343 from domain 961-8 ('961cL-Δaro')
- 961-1 to 961-6 and amino acids 287-315 from domain 961-7 ('961cL-Δcc')
- 961-1 to 961-5 ('961aL')
- 961-1 to 961-4 ('961aL-Δ1')
- 961-1 to 961-3 ('961aL-Δ2')
- 961-1 to 961-2 ('961aL-Δ3')

These thirteen fragments (and sub-fragments thereof missing 1, 2, 3, 4 or 5 amino acids at either or both ends) are preferred (c) and (j) fragments, but they may also be expressed in their own right *i.e.* not in the form of a hybrid protein of the invention. Thus the invention provides a protein comprising

one of these fragments, providing that the protein is not full-length 961 and is not a protein specifically disclosed in reference 1 or 2. This protein may be a fusion protein (*e.g.* a GST-fusion or a His-tag fusion).

Sequences

The invention also provides a protein having an amino acid sequence from SEQ IDs 1 to 24. It also provides proteins and nucleic acid having sequence identity to these. As described above, the degree of 'sequence identity' is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more).

The invention also provides nucleic acid encoding such proteins.

Furthermore, the invention provides nucleic acid which can hybridise to this nucleic acid, preferably under "high stringency" conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

The invention also provides nucleic acid encoding proteins according to the invention.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (*eg.* for antisense or probing purposes).

Nucleic acid according to the invention can, of course, be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

Mixtures

The invention also provides a composition comprising two or more (*i.e.* 2, 3, 4, 5, 6 or 7) of the following proteins:

(1) 287

(2) 741

(3) ORF46.1

(4) 961

(5) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=953

(6) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=919

(7) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=961

The mixture may include one or both of the following proteins, either in combination with two or more of (1) to (7), or in combination with only one of (1) to (7):

(8) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=741

(9) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=936, X_2=741

Where proteins 287 and 741 are included in the mixture (*i.e.* in protein 1, 2, 5, 6, 7 or 8), they may be in the 'ΔG' form. Where protein 961 is included, it is preferably in the form of '961c' in which the N-terminus leader and C-terminus membrane anchor are absent [*e.g.* see refs. 1, 2 & 11].

A preferred mixture comprises the following three proteins:

(1) 961c, preferably $961c_{2996}$ (*e.g.* SEQ ID 31 herein);

(2) NH_2-A-[-X-L-]$_n$-B-COOH, wherein *n* is 2, -X_1- is ΔG287 (preferably $\Delta G287_{NZ}$), -X_2- is 953 (preferably 953_{2996}) lacking its leader peptide, -L_1- is GSGGGG, and -A- comprises a N-terminus methionine (*e.g.* -A- is M or MA) (*e.g.* SEQ IDs 28 & 29 herein); and

(3) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=936 (preferably 936_{2996}), X_2=ΔG741 (preferably $\Delta G741_{MC58}$), L_1=GSGGGG (*e.g.* SEQ ID 30 herein).

The mixtures may also comprise *N.meningitidis* outer membrane vesicles.

Heterologous host

Whilst expression of the proteins of the invention may take place in *Neisseria*, the present invention preferably utilises a heterologous host. The heterologous host may be prokaryotic (*e.g.* a bacterium) or eukaryotic. It is preferably *E.coli*, but other suitable hosts include *Bacillus subtilis, Vibrio cholerae, Salmonella typhi, Salmonenna typhimurium, Neisseria lactamica, Neisseria cinerea, Mycobacteria* (*e.g. M.tuberculosis*), yeast *etc.*

***Vectors* etc.**

The invention provides (a) nucleic acid encoding the proteins described above (b) vectors comprising these nucleic acid sequences (c) host cells containing said vectors (d) compositions comprising the proteins or nucleic acids of the invention, which may be suitable as immunogenic compositions (*e.g.* vaccines) or as diagnostic reagents (e) these compositions for use as medicaments (*e.g.* as vaccines) or as diagnostic reagents (f) the use of these compositions in the manufacture of (1) a medicament for treating or preventing infection due to Neisserial bacteria (2) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisseria bacteria, and/or (3) a reagent which can raise antibodies against Neisseria bacteria and (g) a method of treating a patient, comprising administering to the patient a therapeutically effective amount of these compositions.

Implementing the invention will typically involve the basic steps of: obtaining a first nucleic acid encoding a first protein; obtaining a second nucleic acid encoding a second protein; and ligating the first and second nucleic acids. The resulting nucleic acid may be inserted into an expression vector, or may already be part of an expression vector.

To improve solubility, purification of hybrid proteins may involve the refolding techniques disclosed herein.

Immunogenic compositions and medicaments

The compositions of the invention are preferably immunogenic composition, and are more preferably vaccine compositions. The pH of the composition is preferably between 6 and 7. The pH may be maintained by the use of a buffer. The composition may be sterile.

Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat infection), but will typically be prophylactic.

The invention also provides a composition of the invention for use as a medicament. The medicament is preferably able to raise an immune response in a mammal (*i.e.* it is an immunogenic composition) and is more preferably a vaccine.

The invention also provides the use of a composition of the invention in the manufacture of a medicament for raising an immune response in a mammal. The medicament is preferably a vaccine.

The invention also provides a method for raising an immune response in a mammal comprising the step of administering an effective amount of a composition of the invention. The immune response is preferably protective. The method may raise a booster response.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant); where the vaccine is for prophylactic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

These uses and methods are preferably for the prevention and/or treatment of a disease caused by a *Neisseria* (*e.g.* meningitis, septicaemia, gonorrhoea *etc.*). The prevention and/or treatment of bacterial meningitis is preferred.

Further components of the composition

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, trehalose (WO00/56365) and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. A thorough discussion of pharmaceutically acceptable excipients is available in *Remington's Pharmaceutical Sciences*.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigen, as well as any other of the above-mentioned components, as needed. By 'immunologically

effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Dosage treatment may be a single dose schedule or a multiple dose schedule (*e.g.* including booster doses). The vaccine may be administered in conjunction with other immunoregulatory agents.

The vaccine may be administered in conjunction with other immunoregulatory agents.

The composition may include other adjuvants in addition to (or in place of) the aluminium salt. Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO90/14837; Chapter 10 in ref. 13), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing MTP-PE) formulated into submicron particles using a microfluidizer, (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (2) saponin adjuvants, such as QS21 or Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMS may be devoid of additional detergent *e.g.* WO00/07621; (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (WO99/44636), *etc.*), interferons (*e.g.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), *etc.*; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) *e.g.* GB-2220221, EP-A-0689454; (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions *e.g.* EP-A-0835318, EP-A-0735898, EP-A-0761231; (7) oligonucleotides comprising CpG motifs [Krieg *Vaccine* 2000, 19, 618-622; Krieg *Curr opin Mol Ther* 2001 3:15-24; Roman *et al., Nat. Med.*, 1997, 3, 849-854; Weiner *et al., PNAS USA*, 1997, 94, 10833-10837; Davis *et al., J. Immunol.*, 1998, 160, 870-876; Chu *et al., J. Exp. Med.*, 1997, 186, 1623-1631; Lipford *et al., Eur. J. Immunol.*, 1997, 27, 2340-2344; Moldoveanu *et al., Vaccine*, 1988, 16, 1216-1224, Krieg *et al., Nature*, 1995, 374, 546-549; Klinman *et al., PNAS USA*, 1996, 93, 2879-2883; Ballas *et al., J. Immunol.*, 1996, 157, 1840-1845; Cowdery *et al., J. Immunol.*, 1996, 156, 4570-4575; Halpern *et al., Cell. Immunol.*, 1996, 167, 72-78; Yamamoto *et al., Jpn. J. Cancer Res.*, 1988, 79, 866-873; Stacey *et al., J. Immunol.*, 1996, 157, 2116-2122; Messina *et al., J. Immunol.*, 1991, 147, 1759-1764; Yi *et al., J.*

Immunol., 1996, 157, 4918-4925; Yi *et al.*, *J. Immunol.*, 1996, 157, 5394-5402; Yi *et al.*, *J. Immunol.*, 1998, 160, 4755-4761; and Yi *et al.*, *J. Immunol.*, 1998, 160, 5898-5906; International patent applications WO96/02555, WO98/16247, WO98/18810, WO98/40100, WO98/55495, WO98/37919 and WO98/52581] *i.e.* containing at least one CG dinucleotide, with 5-methylcytosine optionally being used in place of cytosine; (8) a polyoxyethylene ether or a polyoxyethylene ester *e.g.* WO99/52549; (9) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (*e.g.* WO01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (*e.g.* WO01/21152); (10) an immunostimulatory oligonucleotide (*e.g.* a CpG oligonucleotide) and a saponin *e.g.* WO00/62800; (11) an immunostimulant and a particle of metal salt *e.g.* WO00/23105; (12) a saponin and an oil-in-water emulsion *e.g.* WO99/11241; (13) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) *e.g.* WO98/57659; (14) other substances that act as immunostimulating agents to enhance the efficacy of the composition.

Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), *etc.*

Further antigens

Further antigens which can be included in the composition of the invention include:

- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 14, 15, 16, 17 *etc.*
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 18 from serogroup C [see also ref. 19] or the oligosaccharides of ref. 20.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* refs. 21, 22, 23].
- a protein antigen from *Helicobacter pylori* such as CagA [*e.g.* 24], VacA [*e.g.* 24], NAP [*e.g.* 25], HopX [*e.g.* 26], HopY [*e.g.* 26] and/or urease.
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 27, 28].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 28, 29].
- an antigen from hepatitis C virus [*e.g.* 30].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 31 & 32].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 33] *e.g.* the CRM_{197} mutant [*e.g.* 34].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 33].
- a saccharide antigen from *Haemophilus influenzae* B [*e.g.* 19].

- an antigen from *N.gonorrhoeae* [*e.g.* 3, 4, 5].
- an antigen from *Chlamydia pneumoniae* [*e.g.* 35, 36, 37, 38, 39, 40, 41].
- an antigen from *Chlamydia trachomatis* [*e.g.* 42].
- an antigen from *Porphyromonas gingivalis* [*e.g.* 43].
- polio antigen(s) [*e.g.* 44, 45] such as IPV or OPV.
- rabies antigen(s) [*e.g.* 46] such as lyophilised inactivated virus [*e.g.*47, RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 33].
- influenza antigen(s) [*e.g.* chapter 19 of ref. 33], such as the haemagglutinin and/or neuraminidase surface proteins.
- an antigen from *Moraxella catarrhalis* [*e.g.* 48].
- a protein antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 49, 50].
- a saccharide antigen from *Streptococcus agalactiae*
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 50, 51, 52].
- an antigen from *Staphylococcus aureus* [*e.g.* 53].

The composition may comprise one or more of these further antigens.

Where a saccharide or carbohydrate antigen is used, it is preferably conjugated to a carrier protein in order to enhance immunogenicity [*e.g.* refs. 54 to 63]. Preferred carrier proteins are bacterial toxins or toxoids, such as diphtheria or tetanus toxoids. The CRM_{197} diphtheria toxoid is particularly preferred. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [*e.g.* ref. 64], synthetic peptides [*e.g.* 65, 66], heat shock proteins [*e.g.* 67], pertussis proteins [*e.g.* 68, 69], protein D from *H.influenzae* [*e.g.* 70], toxin A or B from *C.difficile* [*e.g.* 71], *etc.* Where a mixture comprises capsular saccharides from both serogroups A and C, it is preferred that the ratio (w/w) of MenA saccharide:MenC saccharide is greater than 1 (*e.g.* 2:1, 3:1, 4:1, 5:1, 10:1 or higher). Saccharides from different serogroups of *N.meningitidis* may be conjugated to the same or different carrier proteins.

Any suitable conjugation reaction can be used, with any suitable linker where necessary.

Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means [32]).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens.

Antigens are preferably mixed with (and more preferably adsorbed to) an aluminium salt (*e.g.* phosphate, hydroxide, hydroxyphosphate, oxyhydroxide, orthophosphate, sulphate). The salt may take any suitable form (*e.g.* gel, crystalline, amorphous *etc.*).

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using proteins antigens in the composition of the invention, nucleic acid encoding the antigen may be used [*e.g.* refs. 72 to 80]. Protein components of the compositions of the invention may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein.

Definitions

The term "comprising" means "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows an alignment of twenty-three sequences for protein 741. These are SEQ IDs 1 to 22 plus the sequence from MC58.

Figure 2 shows an alignment of the NMB1343 sequence from gonococcus (top; SEQ ID 25) and meningococcus (bottom; SEQ ID 26).

Figure 3 shows hybrid and tandem proteins of the invention.

Figure 4 shows 9 domains within 961_{2996}, and Figure 5 shows how these have been manipulated.

MODES FOR CARRYING OUT THE INVENTION

Hybrid proteins – $X_1 = \Delta G287$

In addition to those disclosed in references 1 & 2, seven hybrid proteins with ΔG287 from strain 2996 at the N-terminus were constructed. Eight 287 tandem proteins were also made (see below).

#	*n*	X_1	L_1	X_2	L_2
1	2	ΔG287	-	230	$(His)_6$
2	2		-	936	$(His)_6$
3	2		-	741_{MC58}	$(His)_6$
4	2		-	741_{ET37}	$(His)_6$
5	2		-	$741_{90/18311}$	$(His)_6$
6	2		-	741_{95N477}	$(His)_6$
7	2	$\Delta G287_{nz}$	-	741_{MC58}	$(His)_6$

These proteins were adjuvanted with either Freund's complete adjuvant (FCA) or 3mg/ml alum and used to immunise mice. The resulting sera were tested against various Neisserial strains using the bactericidal assay. Titres using protein #3 were as follows:

Strain (serogroup)	2996 (B)	MC58 (B)	NGH38 (B)	394/98 (B)	44/76 (B)	F6124 (A)
Al hydroxide	8192	32768	8192	>2048	16384	8192
FCA	16384	262144	8192	>2048	>32768	8192

In further experiments using protein #3 adjuvanted with aluminium hydroxide, anti-287 and anti-741 ELISA titres each exceeded 984150 and BCA titres were as follows:

2996 (B)	MC58 (B)	NGH38 (B)	394/98 (B)	44/76 (B)	F6124 (A)	BZ133 (C)
8000	65000	4000	4000	32000	8000	16000

Results obtained after immunisation with proteins disclosed in refs. 1 & 2, tested against the homologous strain, were as follows:

n	X_1	L_1	X_2	L_2	Bactericidal titre		ELISA	
					FCA	Alum	FCA	Alum
2	$\Delta G287_{394/98}$	-	961	$(His)_6$	-	32768	-	>109350
			919		32768	4096	4718	3678
			953		>32768	>16384	1900	6936
			741		16384	2048	232	862
2	$\Delta G287_{2996}$	-	961	$(His)_6$	65536	32768	108627	>109350
			919		128000	32000	11851	2581
			953		65536	-	3834	-
			741		16384	8192	315	4645

Hybrid proteins – X_1 = 961c or 961cL

In addition to those disclosed in references 1 & 2, eight hybrid proteins with either 961c or 961cL (*i.e.* 961c + leader peptide) at the N-terminus were constructed:

#	*n*	X_1	L_1	X_2	L_2
1	2	961c	-	287	-
2	2		-	287	$(His)_6$
3	2		-	230	$(His)_6$
4	2		-	936	$(His)_6$
5	2	961cL	-	287	-
6	2		-	287	$(His)_6$
7	2		-	230	$(His)_6$
8	2		-	936	$(His)_6$

These proteins were adjuvanted with either Freund's complete adjuvant (FCA) or 3.3mg/ml alum and used to immunise mice. The resulting sera were tested against various Neisserial strains using the bactericidal assay. Titres using protein #8 were as follows:

Strain (serogroup)	2996 (B)	MC58 (B)	394/98 (B)	44/76 (B)	F6124 (A)
Al hydroxide	8192	8192	512	1024	<16
FCA	65536	16384	>2048	>2048	8192

Titres obtained after immunisation with 961c–741 [refs. 1 & 2] were as follows:

Strain (serogroup)	2996 (B)	MC58 (B)	394/98 (B)	44/76 (B)	F6124 (A)	BZ133 (C)
Al hydroxide	65536	32768	4096	>32768	16384	>2048
FCA	>16384	262144	4096	>16384	-	>2048

These results could be improved by mixing 961c–741 with ORF46.1 or with ΔG287–919.

Results obtained after immunisation with proteins disclosed in refs. 1 & 2, tested against the homologous strain, were as follows:

n	X_1	L_1	X_2	L_2	Bactericidal titre		ELISA	
					FCA	Alum	FCA	Alum
2	961c	-	ORF46.1	$(His)_6$	32768	1024	>109350	>109350
			741		>16384	8192	>109350	>109350
			936		>32768	8192	>109350	>109350

Hybrid proteins – X_1 = ORF46.1

In addition to those disclosed in references 1 & 2, two hybrid proteins with ORF46.1 at the N-terminus were constructed:

#	*n*	X_1	L_1	X_2	L_2
1	2	ORF46.1	-	936	$(His)_6$
2	2		-	230	$(His)_6$

These proteins were adjuvanted with either Freund's complete adjuvant (FCA) or 3mg/ml alum and used to immunise mice. The resulting sera were tested against the homologous strain using the bactericidal assay and by ELISA.

Results obtained after immunisation with proteins disclosed in refs. 1 & 2 were as follows:

n	X_1	L_1	X_2	L_2	Bactericidal titre		ELISA	
					FCA	Alum	FCA	Alum
2	ORF46.1	-	961	$(His)_6$	8192	8192	21558	>109350
		-	961c	$(His)_6$	8192	128	9020	76545

Hybrid proteins – $X_1 = 230$

In addition to those disclosed in references 1 & 2, four hybrid proteins with 230 at the N-terminus were constructed:

#	*n*	X_1	L_1	X_2	L_2
1	2	230	-	ORF46.1	$(His)_6$
2	2		-	961	$(His)_6$
3	2		-	961c	$(His)_6$
4	2		-	741_{MC58}	$(His)_6$

Hybrid proteins – $X_1 = 936$

In addition to those disclosed in references 1 & 2, seven hybrid proteins with 936 at the N-terminus were constructed:

#	*n*	X_1	L_1	X_2	L_2
1	2	936	-	ORF46.1	$(His)_6$
2	2		-	961	$(His)_6$
3	2		-	741_{ET37}	$(His)_6$
4	2		-	741_{MC58}	$(His)_6$
5	2		-	$741_{90/18311}$	$(His)_6$
6	2		-	741_{95N477}	$(His)_6$
7	2		-	741	$(His)_6$

These proteins were adjuvanted with either Freund's complete adjuvant (FCA) or 3mg/ml alum and used to immunise mice. The resulting sera were tested against various Neisserial strains using the bactericidal assay. Titres using protein #2 were as follows:

Strain (serogroup)	**2996** (B)	**MC58** (B)	**394/98** (B)	**44/76** (B)	**F6124** (A)
Al hydroxide	16384	32768	1024	2048	<16
FCA	65536	65536	>2048	8192	2048 (36%)

Titres using protein #4 were as follows:

Strain (serogroup)	**2996** (B)	**MC58** (B)	**394/98** (B)	**44/76** (B)	**F6124** (A)
Al hydroxide	256	>262144	>2048	32768	8192
FCA	1024	>262144	>2048	>32768	>32768

Titres using protein #7 were as follows:

Strain (serogroup)	**2996** (B)	**MC58** (B)	**394/98** (B)	**44/76** (B)	**F6124** (A)	**BZ133** (C)
Al hydroxide	256	130000	16000	32000	8000	16000

Results obtained after immunisation with proteins disclosed in refs. 1 & 2, tested against the homologous strain, were as follows:

n	X_1	L_1	X_2	L_2	Bactericidal titre		ELISA	
					FCA	Alum	FCA	Alum
2	936	-	741	$(His)_6$	1024	256	1466	5715
			936		>32768	>32768	>109350	>109350

Mixtures of hybrid proteins

Mice were immunised with of three proteins adjuvanted with aluminium hydroxide, either single or in a triple combination: (1) 287_{NZ}–953; (2) 936–741; and (3) 961c. The mixture was able to induce high bactericidal titres against various strains:

	2996 (B)	MC58 (B)	NGH38	394/98 (B)	H44/76 (B)	F6124 (A)	BZ133 (C)	C11 (C)
(1)	32000	16000	130000	16000	32000	8000	16000	8000
(2)	256	131000	128	16000	32000	8000	16000	<4
(3)	32000	8000	—	—	—	8000	—	32000
mix	**32000**	**32000**	**65000**	**16000**	**260000**	**65000**	**>65000**	**8000**
(X)	4000	4000	1000	1000	>4000	1000	4000	n.d.

'–' indicates that this strain contains no NadA gene
(X) was a combination of protein 287 with outer membrane vesicles, for comparison

Looking at individual mice, the mixture induced high and consistent bactericidal titres:

#	1	2	3	4	5	6	7	8	9	10
2996	32768	16384	65536	32768	32768	65536	65536	32768	65536	8192
MC58	65536	32768	65536	65536	65536	8192	65536	32768	32768	65536
394/98	65536	4096	16384	4096	8192	4096	32768	16384	8192	16384

Tandem proteins

Hybrid proteins of the invention can be represented by formula NH_2-[-X-L-]$_n$-COOH. Where all *n* instances of -X- are the same basic protein (either identical, or the same protein from different strains or species), the protein is referred to as a 'tandem' protein.

Twelve specific tandem proteins are:

#	*n*	X_1	L_1	X_2	L_2
1	2	$\Delta G741_{MC58}$	-	741_{MC58}	$(His)_6$
2	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{394/98}$	$(His)_6$
3	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{2996}$	$(His)_6$
4	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{394/98}$	$(His)_6$
5	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{2996}$	$(His)_6$

6	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{394/98}$	-
7	2	$\Delta G287_{2996}$	$(Gly)_6$	$\Delta G287_{2996}$	-
8	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{394/98}$	-
9	2	$\Delta G287_{394/98}$	$(Gly)_6$	$\Delta G287_{2996}$	-
10	2	$\Delta G741_{MC58}$	-	$741_{394/98}$	$(His)_6$
11	2	$\Delta G741_{MC58}$	-	$741_{90/18311}$	$(His)_6$
12	2	$\Delta G741_{MC58}$	-	741_{95N477}	$(His)_6$

Proteins #1 to #5 have all been expressed in soluble form in *E.coli*. Expression levels were between 0.24 and 0.50 mg protein per litre of culture. The tandem proteins were purified and mixed with aluminium phosphate as an adjuvant. Tandem proteins #2, #4 and #5 adsorbed readily to aluminium phosphate; adsorption was less complete for tandem proteins #1 and #3.

Allelic variants – 741

Twenty-two polymorphic sequences of 741 were found (SEQ IDs 1 to 22). These and the MC58 sequence are aligned in Figure 1.

Allelic variants – NMB1343

Using PCR on 42 strains of meningococcus of various serogroups, the gene encoding NMB1343 protein was found in 24/42 and was absent in 18/42 strains (Table 1). The NMB1343 gene was sequenced for 10 of the NMB1343$^+$ strains (Table 1, column 3). The nucleic acid sequence (and thus amino acid sequence SEQ ID 23; GenBank AAF41718) was identical in all 10 strains.

NMB1343 was also detected in two strains of *N.gonorrhoeae* (F62 and SN4). The amino acid sequence from gonococcus is SEQ ID 24. An alignment with the meningococcal sequence is:

```
                 . . . .10 . . . .20 . . . .30 . . . .40 . . . .50
Ng     1:INNLWEISYLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKAT: 50
Nm     1:~~~~~MGNFLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKAT: 45

                 . . . .60 . . . .70 . . . .80 . . . .90 . . . 100
Ng    51:HGPSVKNAVYAHQIETDLYDGCYISTTTDKEIAKKFATSSGIENGYIYVL:100
Nm    46:HGPSVKNAVYAHQIETGLYDGCYISTTTDKEIAKKFATSSGIENGYIYVL: 95

                 . . . 110 . . . 120 . . . 130 . . . 140 . . . 150
Ng   101:NRDLFGQYSIFEYEVEHPENPDEKEVTIRAEDCGCIPEEVIIAKELIEIN:150
Nm    96:NRDLFGQYSIFEYEVEHPENPNEKEVTIRAEDCGCIPEEVIIAKELIEIN:145
```

An alignment of the corresponding nucleotide sequences is shown in Figure 2. This shows that the gonococcal sequence has a 4mer insertion in the 5' region of the NMB1343 gene which causes a frameshift and consequent loss of the 5' methionine residue.

Domain deletion – 961

961 is not present in the *N.meningitidis* serogroup A genome sequence [81], even though the surrounding regions are conserved (>90%) between serogroups A and B. References 11 and 12 disclose polymorphic forms of 961. The gene was found to be present in 91% of serogroup B strains belonging to hypervirulent lineages ET-5, ET-37 and cluster A4, but was absent in all strains of lineage 3 tested. Most of the serogroup C strains tested were positive even if not belonging to hypervirulent lineages. The same was true for the serogroup B strains with serotype 2a and 2b. For serogroup A, one strain belonging to subgroup III was positive whereas the other two strains belonging to subgroup IV-1 were negative. 961 was absent in *N.gonorrhoeae* and in commensal species *N.lactamica* and *N.cinerea*.

Figures 4 and 5 show domains in protein 961.

When the anchor region (domain 9) of protein 961 is deleted ('961cL') and expressed in *E.coli*, the protein is exported in the periplasm and secreted in the supernatant of the culture.

To investigate this further, deletion mutants in the C-terminal region of 961 were constructed (961cL-Δaro, 961cLΔcc, 961aL, 961aL-Δ1, 961aL-Δ2, 961aL-Δ3) on the basis of structural features (deletions of aromatic residues in the cases of 961cΔaro mutant, and of coiled-coil regions for the others). These were analysed for expression and secretion into the periplasm and the supernatant of the culture. In all of these deletion mutants, the protein is produced in large amount, is present in periplasmic fraction, and is released in the supernatant of the culture.

ΔG287 – cross-strain bactericidal activity

287 was cloned for five different *N.meningitidis* serogroup B strains and was manipulated to delete the N-terminus up to the end of the poly-glycine region and to introduce a C-terminal his-tag. This gave five ΔG287 proteins. These were adjuvanted with FCA and used to raise immune sera in mice, which were then tested for bactericidal activity against all five serogroup B strains and also against serogroup A and C strains. Bactericidal titres were as follows:

Protein strain	Sera tested for bactericidal activity against strain *						
	2996	**BZ232**	**MC58**	**1000**	**394/98**	**F6124**	**BZ133**
2996	**16000**	128	4096	4096	1024	8000	16000
BZ232	>8000	**256**	2048	8000	2048	16000	8000
MC58	>8000	64	**>8000**	8000	2048	8000	8000
1000	>8000	64	4096	**8000**	1024	16000	16000
394/98	>16000	128	16000	>2048	**>16000**	-	-

* titres against homologous strain shown in bold

Refolding

To improve the levels of soluble protein for some hybrid proteins, alternative refolding protocols to those disclosed in reference 2 were adopted.

Inclusion bodies (IBs) were isolated as follows:

1. Homogenize cells (5g wet weight) in 25 ml 0.1 M Tris-HCl pH 7, 1mM EDTA, at 4°C using an ultraturrax (10 000 rpm)
2. Add 1.5mg lysozyme per gram cells, mix shortly with an ultraturrax, and incubate at 4°C for 30 min.
3. Use sonication or high-pressure homogenization (French press) to disrupt the cells.
4. To digest DNA, add $MgCl_2$ to a final concentration of 3mM and DNase to a final concentration of 10μg/ml, and incubate for 30 min at 25°C
5. Add 0.5 vol. 60 mM EDTA, 6% Triton X-100, 1,5M NaCl pH7, to the solution, and incubate for 30 min at 4°C.
6. Spin down inclusion bodies by centrifugation at 31000*g* (20 000 rpm) for 10 min, 4°C.
7. Resuspend pellet in 40 ml 0.1 M tris-HCl pH 7, 20mM EDTA, using an ultraturrax
8. Repeat centrifugation step 6.
9. The inclusion body pellet may be used, or stored frozen at –20°C.

Hybrid proteins were expressed in *E.coli* as follows:

Protein	Culture volume (litres)	Flask volume (litres)	Temp (°C)	Final OD_{600}	Inclusion body yield (w/w)
ORF46.1-961-His	1	2	37	1.51	33.2%
ORF46.1-961c-His	1	2	37	1.6	28.3%
961c-ORF46.1His	1	2	37	1.18	23.5%
orf46.1-741 His	5	5	37	12.42	35.2

The pellets were solubilised, refolded, ultrafiltered, dialysed, and protein was then purified:

ORF46.1-961-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of solubilised protein was diluted in 400 ml of refolding buffer (0.1M Tris HCl,1M L-arginine, 2mM EDTA pH 8.2) and incubated for 1 hour at 15°C, resulting in a protein concentration of 5μg/ml. Subsequently, another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10 μg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 130 ml final volume. The

ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24hours against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5 The supernatant isolated after centrifugation was used for His-tag purification.

orf 46.1-961c-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of the solubilised protein was diluted in 400 ml refolding buffer (0.5M Tris HCl,1M L-arginine,2 mM EDTA pH 8.2) and incubated for 1 h at 15°C, resulting in a protein concentration of 5µg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10µg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 150 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for His-tag purification.

961c-orf46.1-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 1 mg/ml. To refold the protein, 2 ml of the solubilised protein was diluted in 400 ml refolding buffer (0.1M Tris HCl,0.5 M L-arginine,2 mM EDTA pH 8.2) and incubated for 1 h at 15°C, resulting in a protein concentration of 5 µg/ml. Subsequently another 2 ml of the solubilized protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 10 µg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 150 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for His-tag purification.

orf46.1-741-His IBs were solubilised as follows: IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 10 mg/ml. To refold, 2 ml of the solubilised protein was diluted in 400 ml of the refolding buffer (0.5M Tris HCl,0.7 M L-arginine,2 mM EDTA pH 7.2) and incubated for 1 h at 15°C, resulting in a protein concentration of

50µg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 100µg/ml. The material was ultrafiltered using a 300 ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30 kDa cut-off (YM30) resulting in 120 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 24h against 10 L of 0.1M Tris HCl pH 8,2 buffer. A second dialysis of 24h against 10 L of 300mM NaCl, 50 mM sodium phosphate pH 8,0 buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5 The supernatant isolated after centrifugation was used for His-tag purification.

Compared with proteins purified as described in ref. 2, bactericidal assay titres were as follows:

Protein	Reference 2		Refolded		
	CFA	Aluminium hydroxide	Aluminium hydroxide	MF59	Aluminium phosphate
ORF46.1-961-His	8192	8192	32768	-	-
ORF46.1-961c-His	8192	128	<64	8192	-
961c-ORF46.1His	32768	1024	16384	-	-
orf46.1-741 His	<4	16	<4	256	-

Similar procedures were used for ORF46.1 to purify the protein from IBs when expressed with no His-tag ('ORF46.1K'):

Protein	Culture volume (litres)	Flask volume (litres)	Temp (°C)	Final OD_{600}	Inclusion body yield (w/w)
orf46.1K	5	5	37	13.7	29.4

IB proteins were resuspended in 4 ml of 6M guanidine HCl, 1 mM EDTA pH 8.5 buffer, to a final protein concentration of 10 mg/ml. To refold, 2 ml of the solubilised protein was diluted in 400 ml of the refolding buffer (0.5M Tris HCl,0.7 M L-arginine,2 mM EDTA pH 7.2) and incubated for 1 hours at 15°C, resulting in a protein concentration of 50µg/ml. Subsequently another 2 ml of the solubilised protein was added and incubated for an additional hour at the same temperature resulting in a final protein concentration of 100µg/ml. The material was ultrafiltered using a 300ml Amicon ultrafiltration cell (8400), applying a 3 bar pressure on an Amicon membrane with a 30kDa cut-off (YM30) resulting in 120 ml final volume. The ultrafiltered material was dialysed using a regenerated cellulose tubular membrane with a 12-14 kDa cutoff (Cellusep – Step bio) for 12h against 10 L of 50mM sodium phosphate, 2mM EDTA, pH 7,2 buffer. A second dialysis of 24h against 10 L of the same buffer was performed. The dialysed material was centrifuged at 22000 rpm for 45 minutes at 4°C in a Beckman centrifuge rotor JA25.5. The supernatant isolated after centrifugation was used for cationic exchange chromatography. The purification was done on a AKTA explorer chromatography

system (Amersham-Pharmacia Biotech) using a 5 ml HiTrap SP sepharose HP column (Amersham-Pharmacia Biotech). The flow rate applied was of 1.5 ml per minute. The column was washed with 35 ml of 50mM sodium phosphate buffer pH 7.2. A linear gradient (0-1 M NaCl) was performed using a 50mM sodium phosphate buffer pH 7.2. The protein eluted in two peaks at 92 mM and 380mM NaCl. The fractions constituting each peak were pooled and respectively named pool 1 and pool 2.

Compared with proteins purified as described in ref. 2, bactericidal assay titres when adjuvanted with aluminium hydroxide were improved from <4 to 1024. The titre using aluminium phosphate adjuvant with the refolded protein was 2048. ELISA titres were as follows:

Protein	Aluminium adjuvant	Elisa (M7)	SBA (2996)
Orf46.1k (pool 1)	Hydroxide 3.3mg/ml	1212	512
	Phosphate 0.6 mg/ml	154	1024
Orf46.1k (pool 2)	Hydroxide 3.3mg/ml	1085	1024
	Phosphate 0.6 mg/ml	250	1024

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

TABLE 1

Strain	1343	Sequence	Strain classification
72/00	+		ET5 B:15:P1.7,13,13a
30/00	+		ET5 B:15:P1.7,16
39/99	+		ET5 C:15:P1.7,16
95330	+		ET5 B:4:P1.15
M4102	+		ET5 nd
MC58(21)	+	+	ET5 B:15:P1.7,16b
BZ169(7)	+	+	ET5 B:NT:P1.16
BZ83(19)	+		ET5 B:15:-.-
CU385	+	+	ET5 B:4:P1.15
220173I	+		ET5 NG:4:P1.15
64/96	+	+	ET5 NG:15:P1.7,16 (carrier)
220173I	+		ET5 B:4:P1.15 (carrier)
ISS1071	+		nd B:15:P1.7,16 (ET5?)
BZ198(2)	+	+	lin.3 B:8:P1.1
980-2543	+	+	lin.3 B:NT:P1.4
16060	+	+	other B:4:P1.14 (carrier)
394-98	+		nd B:4:P1.4 (lin 3?)
ISS1106	+		nd B:4:P1.4 (lin.3?)
BZ133(10)	+	+	sub I B:NT:-.-
S3446	+	+	nd B:14:P1.23,14
ISS1001	+	+	nd B:14:P1.13
241175I	+		other NG:21:P1.16 (carrier)
171274I	+		other NG:15:- (carrier)
66/96	+		other B:17:P1.15 (carrier)
961-5945	-		A4
96217	-		A4
312294	-		A4
90/18311(24)	-		ET37
93/4286(25)	-		ET37
M986	-		ET37
1000(5)	-		other
NGE28(13)	-		other carrier
NGH38(14)	-		other carrier
BZ232(18)	-		other
F6124(23)	-		sub III A:-.-
C11	-		C:-
NMB	-		nd
8047	-		nd
ISS759	-		nd C:2b:P1.2
ISS1113	-		nd C:2:P1.5
65/96	-		nd 4:P1.14
2996(96)	-		nd B:2b:P1.5,2

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CLAIMS

1. A hybrid protein having formula:

$$NH_2\text{-A-[-X-L-]}_n\text{-B-COOH}$$

wherein L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, and *n* is an integer greater than 1, and X is either:

(a) an orf1, orf4, orf25, orf40, orf46.1, orf83, NMB1343, 230, 233, 287, 292, 594, 687, 736, 741, 907, 919, 936, 953, 961 or 983 amino acid sequence;

(b) an amino acid sequence having sequence identity to an amino acid sequence from (a); or

(c) an amino acid sequence comprising a fragment of an amino acid sequence from (a).

2. A hybrid protein having formula:

$$NH_2\text{-A-[-X-L-]}_n\text{-B-COOH}$$

wherein X is an amino acid sequence, L is an optional linker amino acid sequence, A is an optional N-terminal amino acid sequence, B is an optional C-terminal amino acid sequence, *n* is an integer greater than 1, and wherein a first X moiety ($-X_a-$) has one of the following amino acid sequences:

(d) the 446 even SEQ IDs (i.e. 2, 4, 6, ... , 890, 892) disclosed in reference 3.

(e) the 45 even SEQ IDs (i.e. 2, 4, 6, ... , 88, 90) disclosed in reference 4;

(f) the 1674 even SEQ IDs 2-3020, even SEQ IDs 3040-3114, and all SEQ IDs 3115-3241, disclosed in reference 5;

(g) the 2160 amino acid sequences NMB0001 to NMB2160 from reference 7; or

(h) an amino acid sequence disclosed in reference 1 or reference 2,

and a second -X- moiety ($-X_b-$), wherein $-X_b-$ has sequence identity to $-X_a-$ and/or $-X_b-$ comprises a fragment of $-X_a-$.

3. The hybrid protein of claim 1 or claim 2, wherein *n*=2.

4. The hybrid protein of claim 2, wherein $-X_a-$ is an orf46.1, 230, 287, 741, 919, 936, 953, 961 or 983 amino acid sequence.

5. The hybrid protein of claim 2, wherein $X_1, \ldots, X_n$ all have sequence identity to each other.

6. The hybrid protein of any preceding claim, wherein n=2, and wherein the -X- moieties are: ΔG287 and 230; ΔG287 and 936; ΔG287 and 741; 961c and 287; 961c and 230; 961c and 936;

961cL and 287; 961cL and 230; 961cL and 936; ORF46.1 and 936; ORF46.1 and 230; 230 and 961; 230 and 741; 936 and 961; 936 and 741; ΔG741 and 741; or ΔG287 and 287.

7. The hybrid protein of any preceding claim, wherein L has 20 or fewer amino acids.

8. The hybrid protein of any preceding claim, wherein L is a poly-glycine linker.

9. The hybrid protein of any preceding claim, wherein A has 40 or fewer amino acids.

10. The hybrid protein of any preceding claim, wherein B has 40 or fewer amino acids.

11. The hybrid protein of any preceding claim, wherein the -X- moieties have an amino acid sequence found in *N.meningitidis* serogroup B.

12. The hybrid protein of any preceding claim, wherein at least one -X- moiety is a 961 amino acid sequence in which one or more domains has been deleted.

13. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 1 to 38.

14. Nucleic acid encoding a protein of any preceding claim.

15. A composition comprising protein or nucleic acid according to any preceding claim.

16. A composition comprising two or more of the following proteins:

(1) 287

(2) 741

(3) ORF46.1

(4) 961

(5) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=953

(6) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=919

(7) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=961

(8) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=287, X_2=741

(9) NH_2-A-[-X-L-]$_n$-B-COOH, wherein n=2, X_1=936, X_2=741

17. The composition of claim 16, comprising proteins (4), (5) and (9).

18. The composition of claim 17, wherein protein (4) comprises SEQ ID 31, protein (5) comprises SEQ ID 28 or SEQ ID 29, and protein (9) comprises SEQ ID 30.

19. The composition of any one of claims 15 to 18, further comprising:

– a protein antigen from *N.meningitidis*;

– an outer-membrane vesicle (OMV) preparation from *N.meningitidis*;

– a saccharide antigen from *N.meningitidis*;

– a saccharide antigen from *Streptococcus pneumoniae*;

- an antigen from hepatitis A, B or C virus;
- an antigen from *Bordetella pertussis*;
- a diphtheria antigen;
- a tetanus antigen;
- a protein antigen from *Helicobacter pylori*;
- a saccharide antigen from *Haemophilus influenzae*;
- an antigen from *N.gonorrhoeae*;
- an antigen from *Chlamydia pneumoniae*;
- an antigen from *Chlamydia trachomatis*;
- an antigen from *Porphyromonas gingivalis*;
- polio antigen(s);
- rabies antigen(s);
- measles, mumps and/or rubella antigens;
- influenza antigen(s);
- an antigen from *Moraxella catarrhalis*;
- an antigen from *Streptococcus agalactiae*;
- an antigen from *Streptococcus pyogenes*; and/or
- an antigen from *Staphylococcus aureus*.

20. The composition of any one of claims 15 to 19, further comprising a pharmaceutically acceptable carrier.

21. The composition of claim 20 for use as a medicament.

22. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of the composition of claim 20.

FIGURE 1

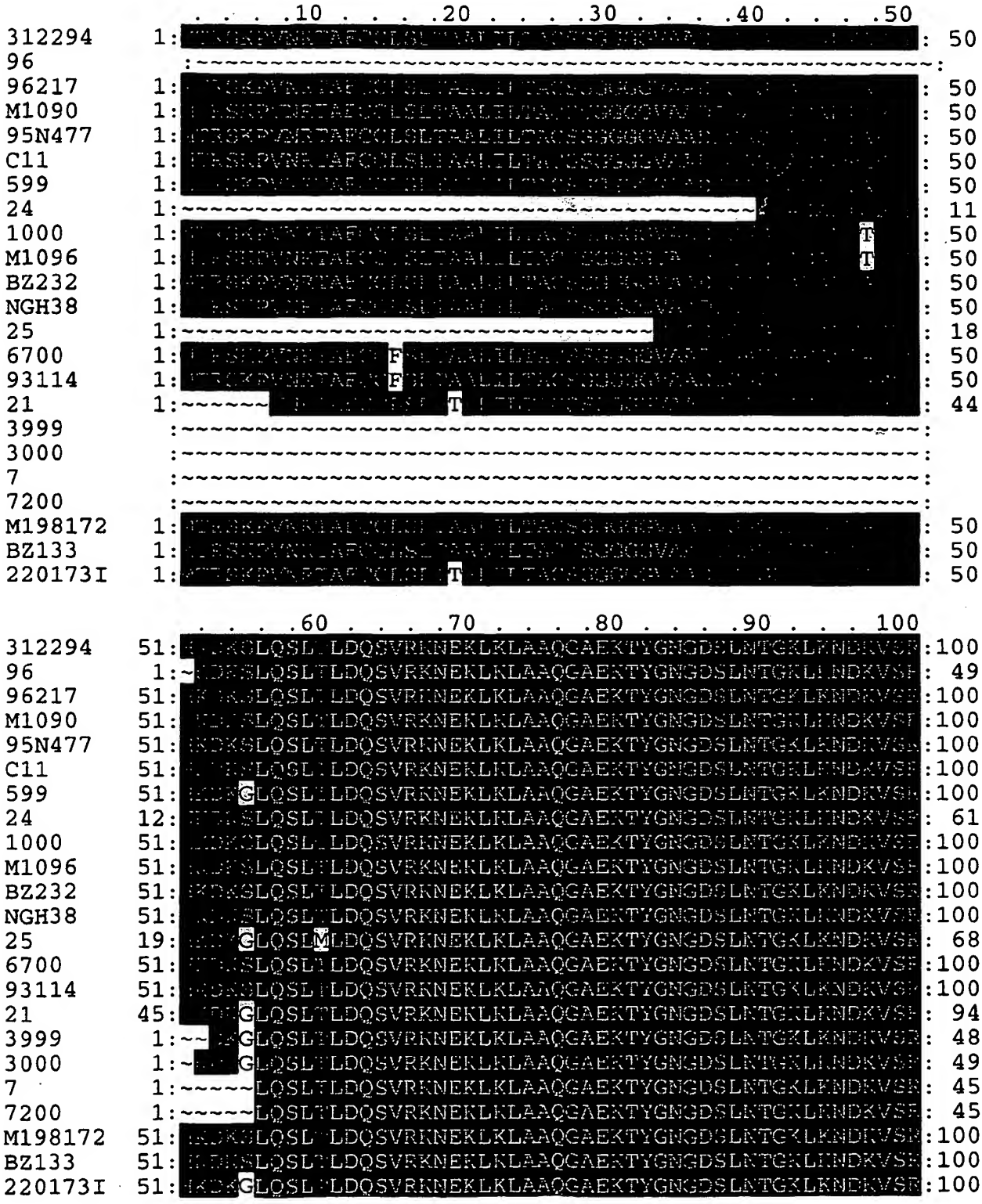


FIGURE 1 CONTD...

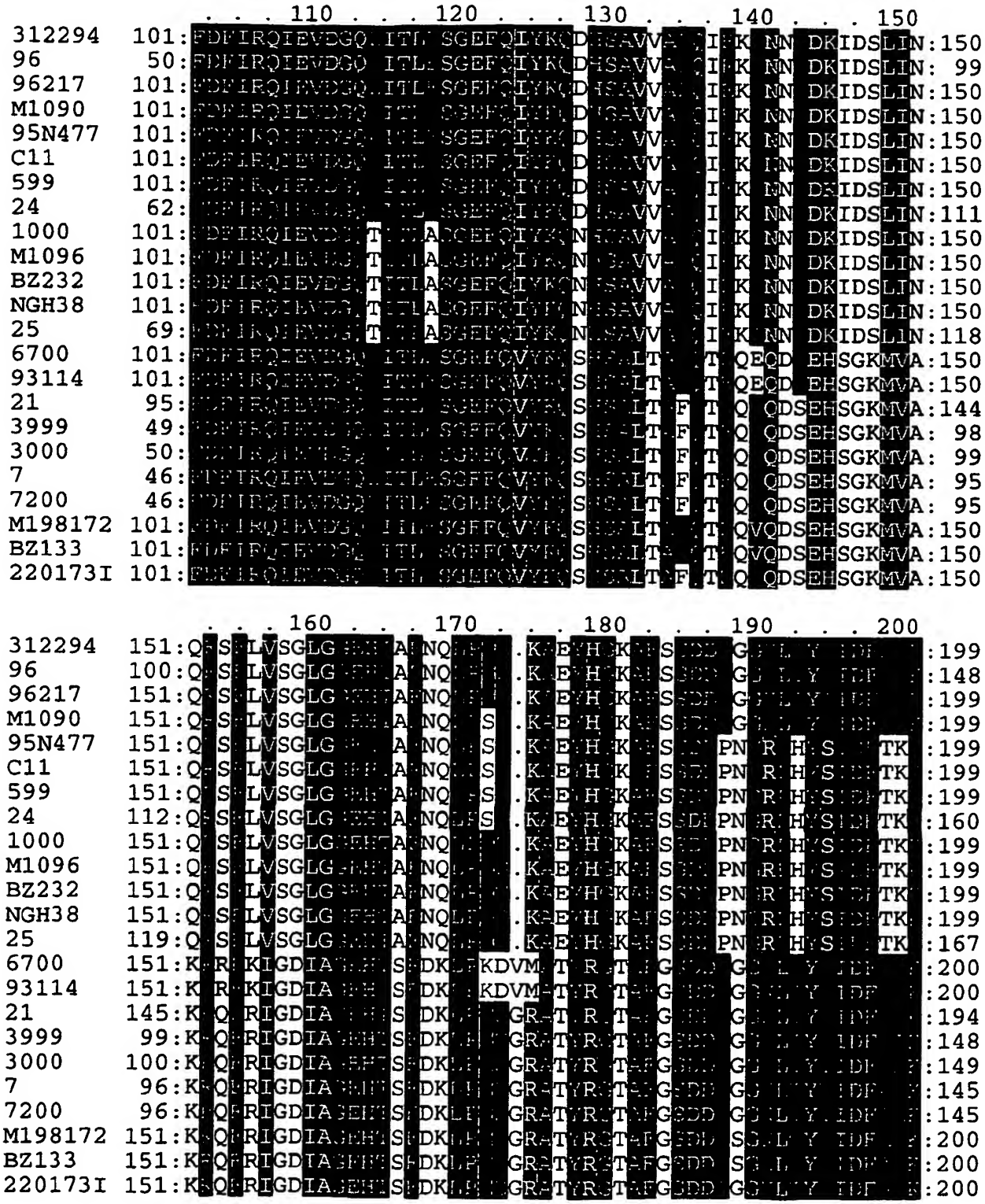


FIGURE 1 CONTD...

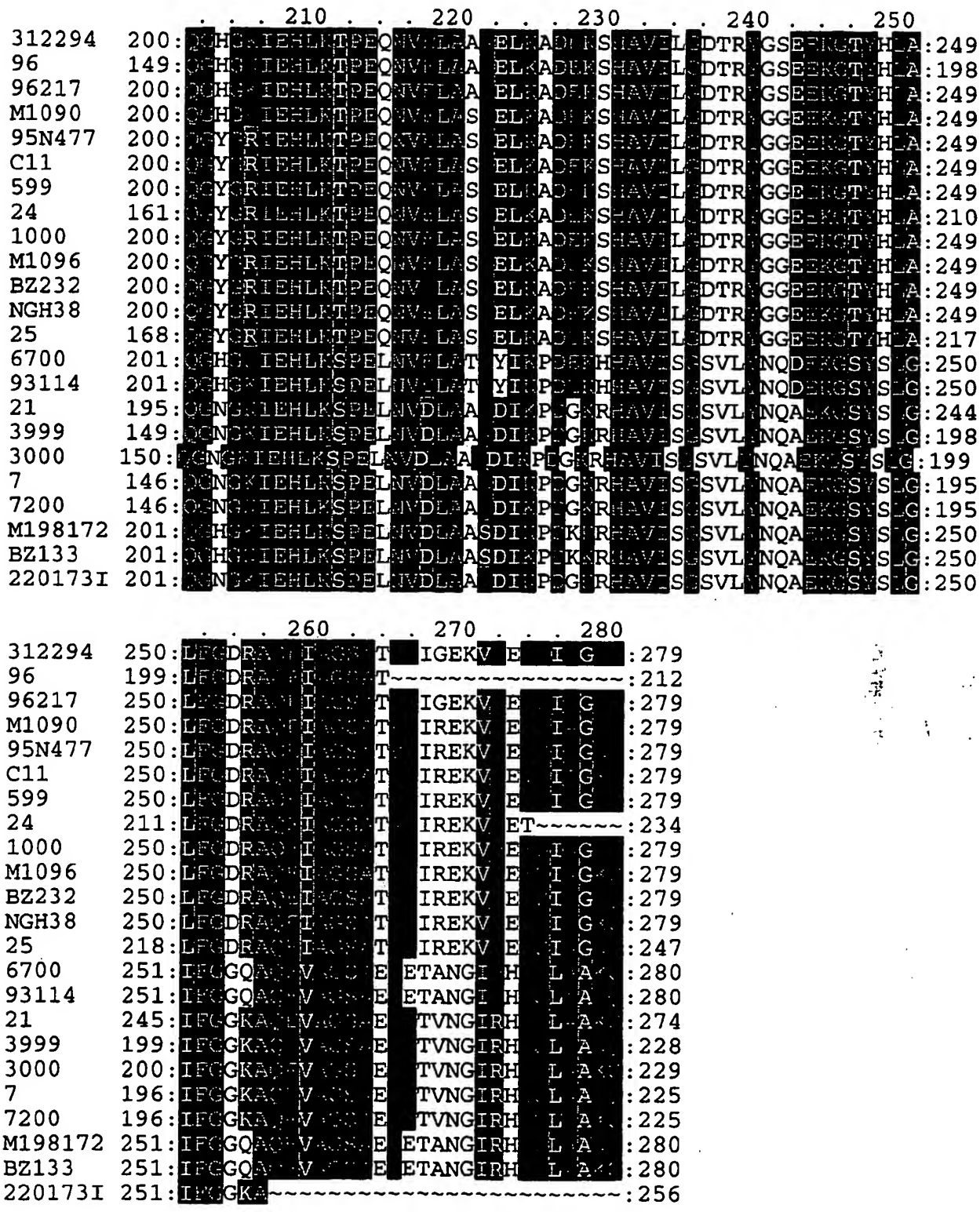


FIGURE 2

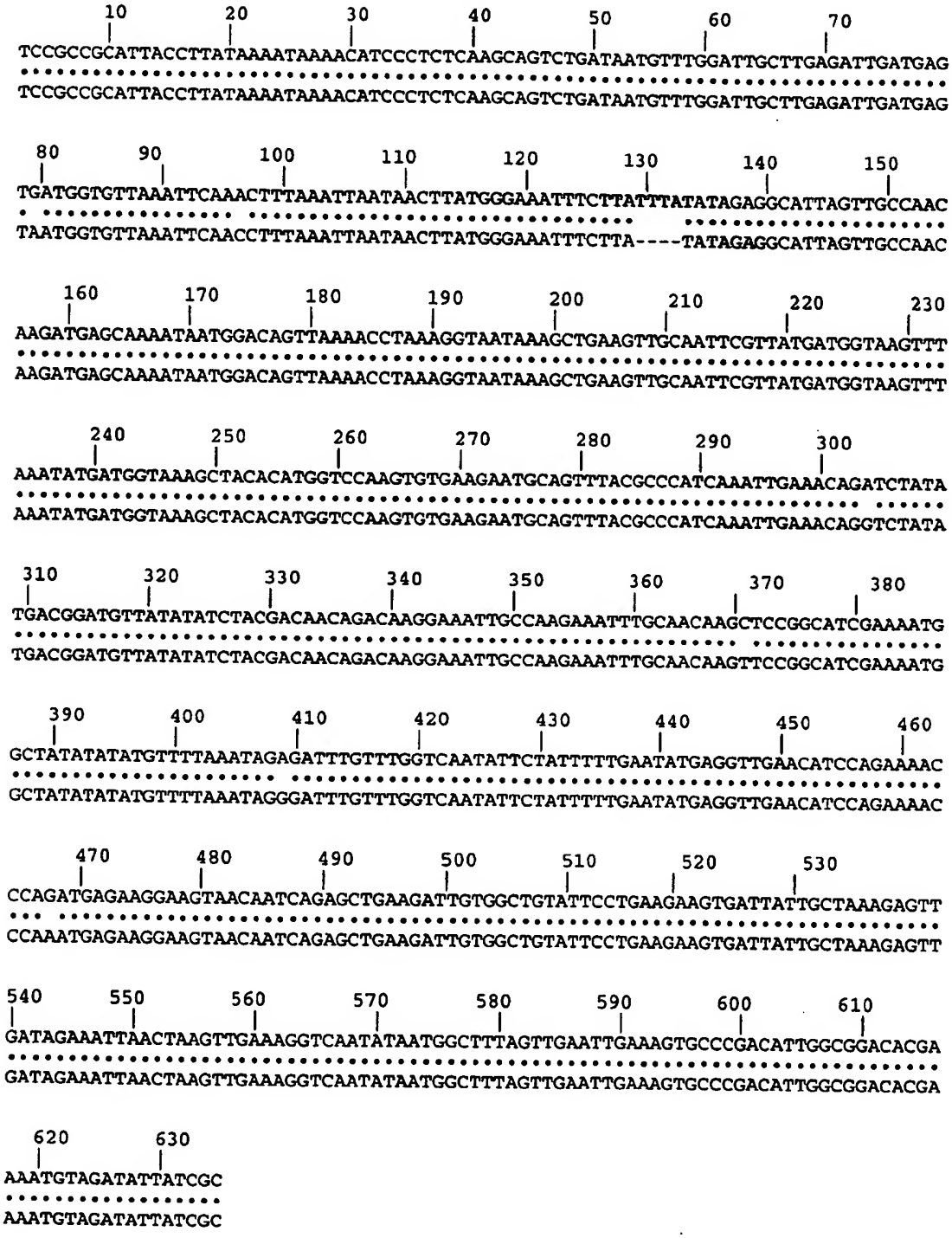

10 20 30 40 50 60 70
TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGATTGATGAG
TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGATTGATGAG
80 90 100 110 120 130 140 150
TGATGGTGTTAAATTCAAACTTTAAATTAATAACTTATGGGAAATTTCTTATTTATATAGAGGCATTAGTTGCCAAC
TAATGGTGTTAAATTCAACCTTTAAATTAATAACTTATGGGAAATTTCTTA----TATAGAGGCATTAGTTGCCAAC
160 170 180 190 200 210 220 230
AAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCAATTCGTTATGATGGTAAGTTT
AAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCAATTCGTTATGATGGTAAGTTT
240 250 260 270 280 290 300
AAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTTACGCCCATCAAATTGAAACAGATCTATA
AAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTTACGCCCATCAAATTGAAACAGGTCTATA
310 320 330 340 350 360 370 380
TGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGCCAAGAAATTTGCAACAAGCTCCGGCATCGAAAATG
TGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGCCAAGAAATTTGCAACAAGTTCCGGCATCGAAAATG
390 400 410 420 430 440 450 460
GCTATATATATGTTTTAAATAGAGATTTGTTTGGTCAATATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAAC
GCTATATATATGTTTTAAATAGGGATTTGTTTGGTCAATATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAAC
470 480 490 500 510 520 530
CCAGATGAGAAGGAAGTAACAATCAGAGCTGAAGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTT
CCAAATGAGAAGGAAGTAACAATCAGAGCTGAAGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTT
540 550 560 570 580 590 600 610
GATAGAAATTAACTAAGTTGAAAGGTCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGA
GATAGAAATTAACTAAGTTGAAAGGTCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGA
620 630
AAATGTAGATATTATCGC
AAATGTAGATATTATCGC

FIGURE 3

ΔG287-919-His
ΔG287-Orf46.1-His
ΔG287-953-His
ΔG287-961-His
ΔG287-230-His
ΔG287-936-His
ΔG287-287-His
ΔG287-287_{nz}-His
ΔG287- 741_{MC58} -His
ΔG287-741_{ET37}-His

ΔG287_{nz}-919-His
ΔG287_{nz}-953-His
ΔG287_{nz}-961-His
ΔG287_{nz}-287-His
ΔG287_{nz}-287_{nz}-His
ΔG287_{nz} - 741_{MC58} -His

ΔG287-919-Orf46.1-His
ΔG287-Orf46.1-919-His
919-287-Orf46-His
Orf46.1-287-919-His

961c-741_{MC58}-His
961c-983-His
961c-Orf46.1-His
961cL-741_{MC58}
961cL-287
961c-230-His
961c-936-His

ΔG741_{MC58}-961c-His
ΔG741_{MC58}-961-His
ΔG741_{MC58}-983-His
ΔG741_{MC58}-Orf46.1-His
ΔG741_{MC58}-741_{MC58}-His
ΔG741_{MC58}-741_{ET37}-His

919-287
953-287
919-Orf46.1-His

Orf46.1-287-His
Orf46.1-919-His
Orf46.1-741_{MC58}-His
Orf46.1-961-His
Orf46.1-961c-His
Orf46.1-983-His
Orf46.1-936-His
Orf46.1-230-His

230-741_{MC58}-His
230-Orf46.1-His
230-961-His
230-961c-His
936-741_{MC58}-His
936-Orf46.1-His
936-961-His
936-741_{ET37}-His

ΔG983-741_{MC58}-His
ΔG983-961c-His
ΔG983-961-His
ΔG983-Orf46.1-His

FIGURE 4

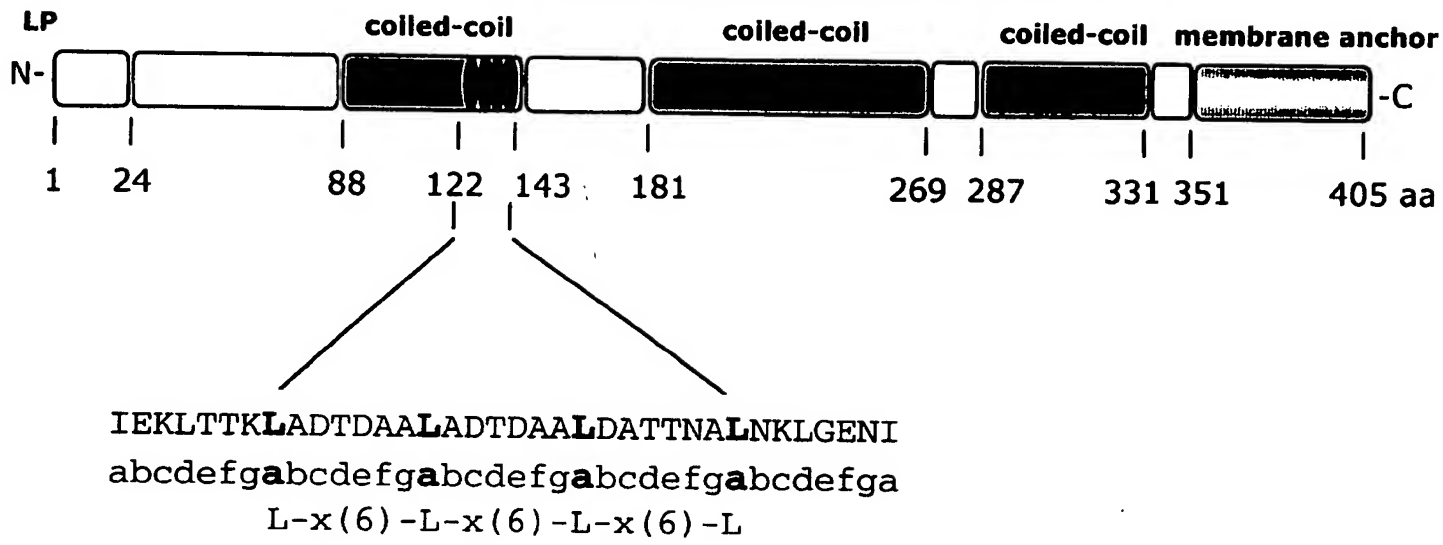
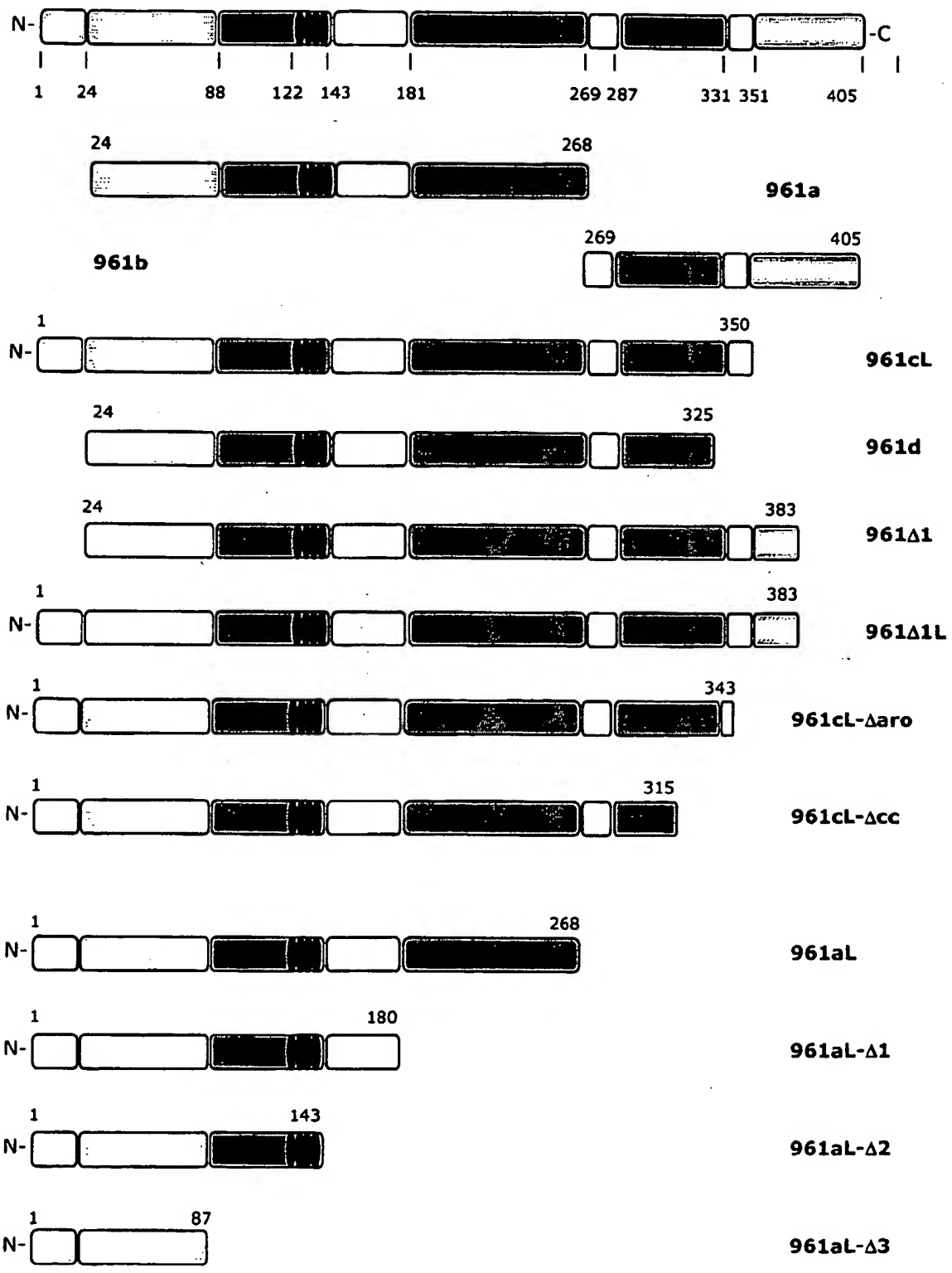


FIGURE 5



SEQUENCE LISTING

SEQ ID 1 – 741 from strain 1000

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTTPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 2 – 741 from strain 220173I (premature stop codon, though reliable sequence)

MTRSKPVNRTAFCCLSLTTALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEHLK
SPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKA

SEQ ID 3 – 741 from strain 90/18311 (incomplete)

GLADALTAPLDHKDKSLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEV
DGQLITLESGEFQIYKQDHSAVVALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGK
AFSSDDPNGRLHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLA
LFGDRAQEIAGSATVKIREKVHET

SEQ ID 4 – 741 from strain L93/4286 (incomplete)

VAADIGAGLADALTAPLDHKDKGLQSLMLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFD
FIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKINNPDKIDSLINQRSFLVSGLGGEHTAFNQLPDG
KAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKTPEQNVELASAELKADEKSHAVILGDTRYGGEE
KGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 5 – 741 from strain 2996

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 6 – 741 from strain 30/00

KDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEF
QVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKL
TYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAG
SAEVKTVNGIRHIGLAAKQ

SEQ ID 7 – 741 from strain 312294

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 8 – 741 from strain 39/99 (incomplete)

DKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQ
VYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLT
YTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGS
AEVKTVNGIRHIGLAAKQ

SEQ ID 9 – 741 from strain 5/99

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 10 – 741 from strain 67/00

MTRSKPVNRTAFCCFSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQEQ
DPEHSGKMVAKRRFKIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTIDFAAKQGHGKIEHLK
SPELNVELATAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGGQAQEVAGSAEVETANGIHHIGLAAKQ

SEQ ID 11 – 741 from strain BZ169

LQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK
QSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTI
DFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEV
KTVNGIRHIGLAAKQ

SEQ ID 12 – 741 from strain 72/00

LQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYK
QSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDAGGKLTYTI
DFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEKGSYSLGIFGGKAQEVAGSAEV
KTVNGIRHIGLAAKQ

SEQ ID 13 – 741 from strain 93/114

MTRSKPVNRTAFCCFSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQEQ
DPEHSGKMVAKRRFKIGDIAGEHTSFDKLPKDVMATYRGTAFGSDDAGGKLTYTIDFAAKQGHGKIEHLK
SPELNVELATAYIKPDEKHHAVISGSVLYNQDEKGSYSLGIFGGQAQEVAGSAEVETANGIHHIGLAAKQ

SEQ ID 14 – 741 from strain 95N477

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 15 – 741 from strain 96217

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELAAAELKADEKSHAVILGDTRYGSEEKGTYHLALFGDRAQEIAGSATVKIGEKVHEIGIAGKQ

SEQ ID 16 – 741 from strain BZ133

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQVQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQGHGKIEHLK
SPELNVDLAASDIKPDKKRHAVISGSVLYNQAEKGSYSLGIFGGQAQEVAGSAEVETANGIRHIGLAAKQ

SEQ ID 17 – 741 from strain BZ232

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 18 – 741 from strain C11

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 19 – 741 from strain M1090

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQIYKQDHSAVVALQIEKIN

NPDKIDSLINQRSFLVSGLGGEHTAFNQLPSGKAEYHGKAFSSDDAGGKLTYTIDFAAKQGHGKIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 20 – 741 from strain M1096

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTTPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 21 – 741 from strain M198/172

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQLITLESGEFQVYKQSHSALTALQTEQVQ
DSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGRATYRGTAFGSDDASGKLTYTIDFAAKQGHGKIEHLK
SPELNVDLAASDIKPDKKRHAVISGSVLYNQAEKGSYSLGIFGGQAQEVAGSAEVETANGIRHIGLAAKQ

SEQ ID 22 – 741 from strain NGH38

MTRSKPVNRTAFCCLSLTAALILTACSSGGGGVAADIGAGLADALTAPLDHKDKSLQSLTLDQSVRKNEK
LKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFIRQIEVDGQTITLASGEFQIYKQNHSAVVALQIEKIN
NPDKIDSLINQRSFLVSGLGGEHTAFNQLPDGKAEYHGKAFSSDDPNGRLHYSIDFTKKQGYGRIEHLKT
PEQNVELASAELKADEKSHAVILGDTRYGGEEKGTYHLALFGDRAQEIAGSATVKIREKVHEIGIAGKQ

SEQ ID 23 – NMB1343 from ten meningococcal strains

MGNFLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKATHGPSVKNAVYAHQIETGLYDGCYIS
TTTDKEIAKKFATSSGIENGYIYVLNRDLFGQYSIFEYEVEHPENPNEKEVTIRAEDCGCIPEEVIIAKE
LIEIN

SEQ ID 24 – NMB1343 from gonococcus

INNLWEISYLYRGISCQQDEQNNGQLKPKGNKAEVAIRYDGKFKYDGKATHGPSVKNAVYAHQIETDLYD
GCYISTTTDKEIAKKFATSSGIENGYIYVLNRDLFGQYSIFEYEVEHPENPDEKEVTIRAEDCGCIPEEV
IIAKELIEIN

SEQ ID 25 – NMB1343 nucleic acid sequence (gonococcus)

TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGAT
TGATGAGTGATGGTGTTAAATTCAAACTTTAAATTAATAACTTATGGGAAATTTCTTATTTATATAGAGG
CATTAGTTGCCAACAAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCA
ATTCGTTATGATGGTAAGTTTAAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTT
ACGCCCATCAAATTGAAACAGATCTATATGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGC
CAAGAAATTTGCAACAAGCTCCGGCATCGAAAATGGCTATATATATGTTTTAAATAGAGATTTGTTTGGT
CAATATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAACCCAGATGAGAAGGAAGTAACAATCAGAG
CTGAAGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTTGATAGAAATTAACTAAGTTGA
AAGGTCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGAAAATGTAGATATTA
TCGC

SEQ ID 26 – NMB1343 nucleic acid sequence (meningococcus)

TCCGCCGCATTACCTTATAAAATAAAACATCCCTCTCAAGCAGTCTGATAATGTTTGGATTGCTTGAGAT
TGATGAGTAATGGTGTTAAATTCAACCTTTAAATTAATAACTTATGGGAAATTTCTTATATAGAGGCATT
AGTTGCCAACAAGATGAGCAAAATAATGGACAGTTAAAACCTAAAGGTAATAAAGCTGAAGTTGCAATTC
GTTATGATGGTAAGTTTAAATATGATGGTAAAGCTACACATGGTCCAAGTGTGAAGAATGCAGTTTACGC
CCATCAAATTGAAACAGGTCTATATGACGGATGTTATATATCTACGACAACAGACAAGGAAATTGCCAAG
AAATTTGCAACAAGTTCCGGCATCGAAAATGGCTATATATATGTTTTAAATAGGGATTTGTTTGGTCAAT
ATTCTATTTTTGAATATGAGGTTGAACATCCAGAAAACCCAAATGAGAAGGAAGTAACAATCAGAGCTGA
AGATTGTGGCTGTATTCCTGAAGAAGTGATTATTGCTAAAGAGTTGATAGAAATTAACTAAGTTGAAAGG
TCAATATAATGGCTTTAGTTGAATTGAAAGTGCCCGACATTGGCGGACACGAAAATGTAGATATTATCGC

SEQ ID 27 – linker

GSGGGG

SEQ ID 28 – preferred ΔG287-953 hybrid

MASPDVKSADTLSKPAAPVVAEKETEVKEDAPQAGSQGQGAPSTQGSQDMAAVSAENTGNGGAATTDKPK
NEDEGPQNDMPQNSAESANQTGNNQPADSSDSAPASNPAPANGGSNFGRVDLANGVLIDGPSQNITLTHC
KGDSCNGDNLLDEEAPSKSEFENLNESERIEKYKKDGKSDKFTNLVATAVQANGTNKYVIIYKDKSASSS
SARFRRSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALR
VQGEPAKGEMLAGTAVYNGEVLHFHTENGRPYPTRGRFAAKVDFGSKSVDGIIDSGDDLHMGTQKFKAAI
DGNGFKGTWTENGGGDVSGRFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYH
ANARFAIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPVANLQSGSQHFTDHLKSADIFDAAQYPD
IRFVSTKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLV
NVGMTKSVRIDIQIEAAKQ

SEQ ID 29 – ΔG287$_{NZ}$-953 hybrid

MASPDVKSADTLSKPAAPVVSEKETEAKEDAPQAGSQGQGAPSAQGGQDMAAVSEENTGNGGAAATDKPK
NEDEGAQNDMPQNAADTDSLTPNHTPASNMPAGNMENQAPDAGESEQPANQPDMANTADGMQGDDPSAGG
ENAGNTAAQGTNQAENNQTAGSQNPASSTNPSATNSGGDFGRTNVGNSVVIDGPSQNITLTHCKGDSCSG
NNFLDEEVQLKSEFEKLSDADKISNYKKDGKNDGKNDKFVGLVADSVQMKGINQYIIFYKPKPTSFARFR
RSARSRRSLPAEMPLIPVNQADTLIVDGEAVSLTGHSGNIFAPEGNYRYLTYGAEKLPGGSYALRVQGEP
SKGEMLAGTAVYNGEVLHFHTENGRPSPSRGRFAAKVDFGSKSVDGIIDSGDGLHMGTQKFKAAIDGNGF
KGTWTENGGGDVSGKFYGPAGEEVAGKYSYRPTDAEKGGFGVFAGKKEQDGSGGGGATYKVDEYHANARF
AIDHFNTSTNVGGFYGLTGSVEFDQAKRDGKIDITIPVANLQSGSQHFTDHLKSADIFDAAQYPDIRFVS
TKFNFNGKKLVSVDGNLTMHGKTAPVKLKAEKFNCYQSPMAKTEVCGGDFSTTIDRTKWGVDYLVNVGMT
KSVRIDIQIEAAKQ

SEQ ID 30 – 936-ΔG741 hybrid

MKPKPHTVRTLIAAIFSLALSGCVSAVIGSAAVGAKSAVDRRTTGAQTDDNVMALRIETTARSYLRQNNQ
TKGYTPQISVVGYNRHLLLLGQVATEGEKQFVGQIARSEQAAEGVYNYITVASLPRTAGDIAGDTWNTSK
VRATLLGISPATQARVKIVTYGNVTYVMGILTPEEQAQITQKVSTTVGVQKVITLYQNYVQRGSGGGGVA
ADIGAGLADALTAPLDHKDKGLQSLTLDQSVRKNEKLKLAAQGAEKTYGNGDSLNTGKLKNDKVSRFDFI
RQIEVDGQLITLESGEFQVYKQSHSALTAFQTEQIQDSEHSGKMVAKRQFRIGDIAGEHTSFDKLPEGGR
ATYRGTAFGSDDAGGKLTYTIDFAAKQGNGKIEHLKSPELNVDLAAADIKPDGKRHAVISGSVLYNQAEK
GSYSLGIFGGKAQEVAGSAEVKTVNGIRHIGLAAKQ

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MATNDDDVKKAATVAIAAAYNNGQEINGFKAGETIYDIDEDGTITKKDATAADVEADDFKGLGLKKVVTN
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KIDEKLEAVADTVDKHAEAFNDIADSLDETNTKADEAVKTANEAKQTAEETKQNVDAKVKAAETAAGKAE
AAAGTANTAADKAEAVAAKVTDIKADIATNKDNIAKKANSADVYTREESDSKFVRIDGLNATTEKLDTRL
ASAEKSIADHDTRLNGLDKTVSDLRKETRQGLAEQAALSGLFQPYNVG